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High-resolution electrochemical STM of redox metalloproteins

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Abstract

Electrochemical studies of redox active metalloproteins have become an increasingly fruitful area of study in recent years, particularly with the single-molecule resolution capability of electrochemical scanning tunnelling microscopy (EC-STM) which provides both imaging and current-voltage spectroscopy under bipotentiostatic control. In this review, some of the most exciting advances in recent years are outlined, and directions for future research are considered.

Keywords: STM, Molecular electronics, Single-molecule break junction, Molecule-electrode interfaces, Electrochemical gating.

Introduction

This review centres on studies of redox active metalloproteins using the technique of electrochemical-scanning tunnelling microscopy (EC-STM). Its purpose is primarily to give a snapshot of recent research in the area, rather than an overarching description of the development of EC-STM, although a number of seminal works must be mentioned. For underpinning background material, both experimental and theoretical, the reader is therefore referred to the existing extensive literature. See, for example, the following (non exclusive) references [1–9] on EC-STM and related techniques. However, it is appropriate first to give a summary of essential concepts.

Experimental techniques

The study of single small organic molecules as well as proteins has seen enormous progress in recent years, following the invention [10] of scanning tunnelling microscopy (STM) and scanning probe techniques generally, to enable a host of physical properties (including electronic, electrical, mechanical and optical) to be examined on surfaces at the atomic scale. Electrochemical STM was developed soon after [11] as a powerful probe of single molecules electrochemistry, allowing not just simple molecular imaging, but current-voltage ($I - V$) characteristics of molecules to be examined when electrochemically 'gated' by a third electrode. The EC-STM technique is a combination of a simple STM with an electrochemical control circuit, outlined in the schematic of figure 1.

In the STM part, an electrical bias V_{tip} (typically 10–100 mV) is applied between the STM tip (which is insulated apart from the extreme end to minimise ionic currents) and the sample to be studied. A quantum mechanical tunnel current I flows across the gap between tip and sample. In this case the sample comprises a carefully prepared, suitably flat (ideally crystalline), conducting surface (often a metal such as gold) with attached individual protein molecules. Images are normally obtained by raster scanning the tip across the surface in constant-current

mode, i.e. under computer feedback to control the height z of the tip above the sample, such that the tunnel current is a chosen fixed value 'set point' (typically a few nA or less). Since the current - distance relationship is exponential in nature $I(z) \sim \exp(-\beta z)$ where β is typically around 10nm^{-1} , a very precise and stable control of z is required.

To further examine the electrical properties of the molecules, rather than just their location and apparent height, a number of different approaches can be used. Conceptually, the simplest is to locate the tip above a molecule and measure the tunnel current as a function of applied tip bias (with feedback turned off) to produce an I versus V_{tip} curve. This can be done for different z . However, long term stability of the instrumentation might not always be sufficient to fix the tip position to do more than a few measurements; in this case a common approach is to perform multiple imaging for a range of set points and bias.

Sometimes, the nature of the surface and molecules (a rough surface, delicate molecules, weak attachment) is not conducive to good imaging, but valuable information can nevertheless be extracted. By measuring the current as the STM tip is repeatedly brought near to (or even in contact with – the 'break junction' or BJ method) the surface and withdrawn again – called the $I(z)$ or $I(s)$ method – it is possible to detect molecular attachment between tip and sample, and hence find the molecular conductivity [12, 13]. Repeating with a range of different tip bias enables full current-voltage characteristics to be determined [14]. These experiments are not simple; it is necessary to analyse many $I(z)$ curves and perform careful statistical analysis on them. Early experiments tended to select curves 'by eye', but more robust data selection techniques [15] are undoubtedly the way forward. There are also several variations of these STM type measurements in which the pulling rate between tip and sample is varied or the bias is changed during the $I(z)$ trace [16].

Another important variation, introduced several years ago [17, 18], is the $I(t)$ method in which an STM tip with fixed bias is located close above a molecule. Again, low drift is essential

for the reliable application of this method and interpretation of the results. Precise details of the measurement (in particular bias, and tip conditioning) also influence the results; it is necessary to have a stable STM tip, and not too high a bias, to get clear reproducible junction formation.

The electrochemical part is based on a potentiostat, which is used to control a potential V of the electrolyte solution with respect to the working electrode (WE) or substrate and measure the resulting current I_m flowing to a counter electrode (CE). Similar to a four-terminal measurement used to eliminate contact effects in determining a resistance, the potentiostat uses a separate current path to the path where the potential is measured. This both prevents instabilities in the current and allows measurement with respect to a reference electrode (RE) maintained at equilibrium. Importantly, in combination with the STM, the potentiostat thus allows *electrochemical gating* of redox active protein molecules which are deposited on the substrate. This is analogous to electrostatic gating of the channel in a field effect transistor (FET). The electrolyte provides electrostatic coupling to the applied external potential although, as it depends on the exchange of charge between ions in solution and the molecule of interest, electrochemical gating is inherently slower than electrostatic gating as it is limited by diffusion of ions. The electrochemical environment of a single protein molecule may also be influenced by the physical presence of the STM tip. One feature actually in favour of electrochemi-

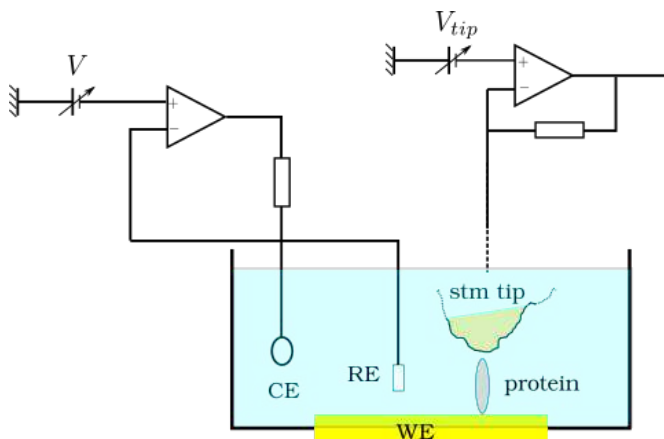


Figure 1: Schematic of EC-STM. In this implementation a (bi)potentiostat controls the potentials on both the STM tip and the surrounding solution with respect to the grounded working electrode.

cal gating [19] is that, with a short Debye screening length, any charge on the molecule in the EC-STM set up is screened by ions of the solution; the effective gate potential environment of the molecule is thus arguably better controlled than for an electrostatic gate, despite the remoteness of counter and reference electrodes. On the other hand, the polarisation of the effective molecule-ion may influence electronic properties.

Outline theoretical description

Electron motion across any molecule which bridges two electrodes (i.e. STM tip and sample) can often be divided into two,

non exclusive, limits [20, 21] where the electron can (i) tunnel directly between the contacts but mediated by the molecule or (ii) tunnel onto the molecule and off again, but with an energy relaxation (due to inelastic phonon scattering) as it resides on the molecule. The first limit describes coherent tunnelling (maintaining phase information) while the second is incoherent. To ascertain which is the dominant mechanism in any particular molecular system requires more information than simple $I - V$ characteristics. This may be provided for example by examining the length dependence of conductance of a homologous series of molecules such as alkanedithiols [22, 14], by measuring temperature dependence, or by examining gate bias dependence. (Hopping transport, which can dominate in longer molecules, is not considered here.)

Reference [23] presents a treatment of coherent electron tunnelling between metallic contacts mediated by a single molecular level, which is commonly the case for small organic molecules. The electrochemical potentials (Fermi levels) of the contacts can be on resonance (energetically aligned with) or off resonance with the molecular level. (The latter picture is in fact not essentially distinct from simple tunnelling, if a non-rectangular barrier is admitted. A Simmons-type tunnelling, assuming a rectangular barrier, is usually sufficient to describe off-resonant tunnelling.) Figure 2 illustrates this model when the relevant energy level is a LUMO of an isolated molecule, which is unoccupied when close to the electrodes. In the lower figure, with a shift of levels due to gating, an electron can tunnel onto the LUMO from a filled state of the same energy in contact 1, and tunnel off again to an empty state in contact 2. This should be viewed (and usually is, for small organic molecules) as a single quantum step. The LUMO level will in general be partially occupied (reduced) since it actually has an intrinsic energy width Γ governed by the coupling strength between the molecule and contacts - the DOS is no longer a delta function. (The relevant wave functions are delocalised over the molecule and overlap the contacts. Γ can be related to the lifetime τ of the state through the uncertainty principle $\tau = \hbar/\Gamma$.) A two-step tunnelling will occur if vibrations in the molecule interact with the electron (destroying phase information, and possibly lowering or raising its energy) during the tunnelling time; if τ is long (weak coupling) then a two-step process is more likely.

In the case of a redox active protein molecule however, as is established by classical electrochemistry studies of electron transfer in proteins, it is further essential to consider both thermally induced fluctuations of the molecular energy levels (internal vibrations and surrounding solvent fluctuations) and the energy of the structural change or 'reorganisation' of the molecule between oxidised and reduced states. EC-STM data has thus been widely and successfully described through the Kuznetsov-Ulstrup (KU) model [24, 7, 25] of *electron transfer*. In this model, near the equilibrium potential a two-step process occurs where both the oxidized and reduced forms of a molecule contribute to the current flow between tip and substrate. Thermal fluctuations effectively result in *probability distributions* for transfer between oxidised and reduced states (arising from the Boltzmann factor and an assumed quadratic dependence of these states' energy levels on configuration coordinate of

the molecule) written as the oxidised (or reduced) distributions $D_{ox}(E)$ (or $D_{red}(E)$). The nature of the model yields normalised

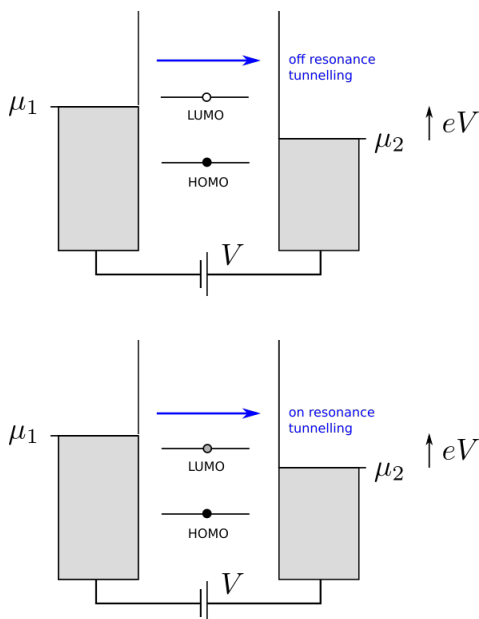


Figure 2: Schematic of electron tunnelling between an STM tip (positive bias V , right) and substrate (left) as mediated by a molecular energy level. The vertical scale is energy. The horizontal scale suggests the contact positions as well as the density of states (DOS). The DOS is shown as constant for the metals and a delta function for the molecular levels. (The molecular levels actually have an intrinsic energy width Γ due to coupling with the metallic states.) Electrochemical potentials μ_1 and μ_2 are separated by eV due to the bias. At zero bias, the metals' Fermi levels are aligned within the HOMO-LUMO gap, while with bias the molecular levels are lowered by $eV/2$ if symmetrically coupled to the contacts. Open (closed) circles indicate unoccupied (occupied) molecular states. Top: The closest molecular level (LUMO in this example) lies above μ_1 , but a current can flow through the broadened tail state of the LUMO. Bottom: The LUMO lies between μ_1 and μ_2 , leading to enhanced current.

Gaussian distributions of width $\sqrt{2kT\lambda}$ where λ is the reorganisation energy. The process of electron transfer might thus be viewed [26–29] as proceeding (for example) through an empty molecular level which momentarily enters the energy window between μ_1 and μ_2 . While the level is in this energy window, a degree of (coherent or incoherent) tunnelling can proceed, but when reorganisation of the protein occurs from the effect of the now (at least partially) reduced level, the level will relax (by an amount 2λ) to take it below the energy window. Fluctuations can take the now fully reduced level back into the energy window at some stage to allow further tunnelling. This picture has been developed into a quantitative analytic description of the $I - V$ characteristics of tunnelling through a redox protein.

Current developments

The power of STM and related techniques is in obtaining single molecule data, which is lost in bulk ensemble studies, and in opening the way to observing dynamical behaviour, revealing a more fundamental understanding of general molecular interactions. Although proteins which are not redox active can be examined with STM [30], there has been extensive use

of EC-STM for gating studies of metalloproteins. Early seminal experiments on the blue single-copper redox active protein azurin [31–33] demonstrated electrochemical gating at the single molecule level. Much work has since centred on this protein, which is robust and can attach to Au surfaces through cysteine residues. For instance, the BJ method in EC-STM was used [34] and confirms the gating observations of the earlier imaging studies. In a recent development, single molecule redox events switching events [35] have been reported for azurin, over an accessible time scale. These are challenging experiments which depend on variation of experimental parameters (sample potential with respect to redox potential in this case) and careful interpretation. Evidence for control of single azurin molecule conductance has even been obtained [36] through the application of voltage pulses. This raises the question of the degree of molecule-substrate coupling and provides further support to the two-step model of electron transfer.

Apart from the possibilities opened up by *time*-resolved measurements, the question naturally arises as to the maximum *spatial* resolution attainable on protein molecules. EC-STM imaging experiments on proteins generally reveal rather featureless structures, although evidence of sub-molecular features were in fact observed [37] in azurin several years ago. Recently, rather detailed sub-molecular features of single streptavidin proteins in solution have been resolved with STM [38], where advantage was taken of carefully optimised conditions. It would be extremely interesting to get improved sub-molecular observation of redox site(s) under electrochemical control.

The nature of the contact between any molecule and surface is well known to be crucial to the conductance measured in STM. Much work has depended on naturally occurring cysteine residues which link to a metal such as gold through a thiol bond. An interesting method to control protein-substrate linking is offered through protein engineering and has been used [39] to attach *cyt-b562* to gold surfaces in specifically chosen orientations. Such methods should be extended to a broader range of proteins, linkers, and substrates in future. It is also worth noting that, if a protein molecule is intimately (covalently) linked to both the STM tip and substrate, strong electronic coupling of molecular levels to the continuum of metallic levels may also be important, as suggested by a number of authors [37, 39, 29, 40].

Theoretical development of EC-STM is also not static. The consensus description is the KU two-step model of charge transfer to describe EC-STM for redox-active molecules. However, Bâldea [41] has considered an alternative approach, perhaps valid for strong tip and substrate coupling, applying instead a Newns-Anderson framework to detailed $I - V$ measurements on azurin which were originally fitted by the authors using the two-step formula. Although both models have a number of adjustable parameters, Bâldea's claim was the use of consistent parameters for different gate biases (reduced and oxidised molecules) to describe the results quantitatively. Interestingly, a suppression of the solvent reorganisation in the nanogap compared to bulk solution was deduced. Further theoretical work should be of interest here.

In any case, the detailed role of the complex surrounding medium is also increasingly being focussed upon in STM-based

experiments [42–45] as well as EC-STM [46]. In terms of general effect on molecular conductivity, local water molecules or ions can both influence the average local electrostatics (shifting molecular levels) and have important dynamical effects. The local structure of water molecules at interfaces is, for example, well known to be partly ordered. Recently, Matyushov *et al* [47–50] have explored how experimentally rather smaller reorganisation energies than expected from atomistic simulations might be explained, examining the case [47] of cytochrome *c*. They extend the Marcus/Gerischer model to distinguish between two types of reorganisation energy representing medium polarisation and (a typically large) thermal fluctuation contribution, the latter being linked to a heterogeneous region surrounding the protein cofactor. The arguments are interesting and would perhaps apply *a fortiori* to the local geometry of the EC-STM set up. Recently, although not directly applicable to proteins, the application of non-aqueous electrochemical gating using ionic liquids and small redox-active molecules has been particularly revealing [51, 19] in understanding the effect of the surrounding medium.

The general environment of a protein is intimately linked to its overall physical properties. References [1] and in particular [52, 53] include good discussions of how the various processes of electron flow between a protein and its environment (electrode or electrolyte) might be categorised, and point to the distinction between mechanisms involved of ‘electron transport’ (essentially, protein-mediated electron current between two adjacent metallic contacts) and ‘electron transfer’ (electrons moving between donor and acceptor sites within a protein, or between a surrounding electrolyte and redox centre in the protein). In all cases however, the driving mechanism is a difference in the electronic electrochemical potential μ , which in EC-STM is tuned by the applied bias between tip and substrate.

Finally, although not the focus of the present review, it is worth noting that electrochemical atomic force microscopy (EC-AFM) measurements can yield interesting information complementary to that of EC-STM since they allow knowledge and control of the force acting between tip and sample, as well as providing reliable height measurement of a protein. For example single molecule redox active Cu-azurin shows evidence [54] of electrochemical modulation of its height, attributed to conformational changes. This is perhaps not surprising in view of the importance of the metal site to protein stability [55].

Progress towards functional devices

A major driving force behind research into functional biomolecules is for active sensing components in device applications for bioelectronics [1, 56, 53]. Due to their inherent molecular function and exquisite recognition properties, protein molecules have become a particular focus [57, 52, 58, 59] in recent years. Proteins which are electrically active are of obvious potential for electronic sensing – if they can be linked to external circuitry. Furthermore, creation of single (or few) molecule devices can provide temporally-resolved information which is lost in bulk ensemble studies, allowing a more fundamental understanding of molecular interactions and behaviour such as transient motions and intermediate states.

To build a protein-based bioelectronic device there are two important prerequisites:

(i) Robust, reproducible and directed physical linking of protein molecules to an electronically active surface (typically a semiconductor). This means that the device can be reliable, long-lived and give a consistent transduction signal. Consistency means that it is possible to distinguish between the subtle changes of signal that would otherwise be masked by random variation.

Although thiol end groups, widely applied in single molecule studies, may occur naturally or be introduced into proteins as a direct means of attachment to metal contacts [39, 60], it is less straightforward to contact the technologically important materials silicon, GaAs or – our focus here – carbon allotropes. Functionalisation of nanoscale devices based on graphene and carbon nanotubes (CNTs) provides an important step in the development of single/few molecule electronic components and miniaturised bio/chemical sensors [61, 58, 62].

(ii) A change in the electronic or morphological properties of the protein, arising from a biorecognition event, should yield an adequate transduction signal. The great sensitivity of the electron network in CNTs and graphene to surface electrostatics makes them especially favourable, and single-walled CNT field-effect transistors are an attractive platform for protein-based biosensors [63, 64].

Much has been learnt about creating biologically active hybrid materials from proteins and CNTs or graphene. Passive adsorption (mostly through hydrophobic interactions) is commonly used to create these interfaces, but while this interaction is intimate, it is also weak and ill defined. An obstacle to achieving a strong and stable linkage with good electrostatic communication between a protein and the electronically active sp^2 system of CNTs is their inherent chemistry. Covalent attachment is the preferred route but there is no inherent compatible chemistry in either the protein or CNT. Thus CNTs are usually first chemically oxidised to introduce the required reactive groups (e.g. ketone, carboxyl or alcohol). Oxidation is difficult to control, leading to wide-scale perturbation of bond π and σ networks that give CNTs their conductance characteristics. Additionally, to functionalise oxidised CNTs with protein, further chemical modification is required to add appropriate linking groups. Such linking groups increase spacing between the CNT and protein and generally rely on reactions with primary amine groups such as lysine amino acids. Lysine residues are common in proteins and are commonly found distributed across the protein surface. Thus, as well as losing intimacy, a single defined and optimal protein interface with CNTs or graphene is generally difficult to achieve. Future advances are thus likely to depend on novel approaches like protein engineering to achieve directed covalent linking. There is also potential to modify protein properties to allow detection, via binding, of a chosen molecular type.

Concluding remarks

High resolution (single or a few molecules) studies of redox active metalloproteins have advanced rapidly in recent

years, driven by interest in understanding basic mechanisms, huge potential for biosensors, and technological advances in measurement. Time-resolved behaviour of single protein molecules under electrochemical control are becoming technically feasible, potentially providing statistics of switching, and may lead to much deeper insight of the nanoscale electron transfer mechanism. Theoretical approaches continue to develop in the areas of (i) electrostatic details around the STM tip and (ii) tunnelling/transfer to include all relevant time scales correctly (iii) the degree of electronic coupling between tip/molecule/substrate. Biosensor applications still require much development. One major obstacle is in going beyond research experiments to make scalable devices. Graphene is an obvious platform here, but there have been promising advances [65] in creating arrays of selected CNTs for FET devices. There is no doubt that nanoscale studies of redox active metalloproteins promise fascinating results for years to come.

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References and recommended reading

- [1] Alessandrini A and Facci P. Electron transfer in nanobiodevices. *Eur Polym J*, **83**:450–466, 2016.
- [2] Nichols RJ and Higgins SJ. Single Molecule Nanoelectrochemistry in Electrical Junctions. *Acc Chem Res*, **49**(11):2640–2648, 2016.
- [3] Mathwig K, Aartsma TJ, Canters GW, and Lemay SG. Nanoscale Methods for Single-Molecule Electrochemistry. *Annu Rev Anal Chem*, **7**(1):383–404, 2014.
- [4] Artés JM, López-Martínez M, Díez-Pérez I, Sanz F, and Gorostiza P. Nanoscale charge transfer in redox proteins and DNA: Towards biomolecular electronics. *Electrochim Acta*, **140**:83–95, 2014.
- [5] Yagati AK, Min J, and Choi JW. Electrochemical Scanning Tunneling Microscopy (ECSTM) From Theory to Future Applications. In *Mod. Electrochem. Methods Nano, Surf. Corros. Sci.*, chapter 3. InTech, 2014.
- [6] Salvatore P, Zeng D, Karlén KK, Chi Q, Wengel J, and Ulstrup J. Electrochemistry of single metalloprotein and DNA-based molecules at Au(111) electrode surfaces. *ChemPhysChem*, **14**(10):2101–2111, 2013.
- [7] Zhang J, Kuznetsov AM, Medvedev IG, Chi Q, Albrecht T, Jensen PS, and Ulstrup J. Single-molecule electron transfer in electrochemical environments. *Chem Rev*, **108**(7):2737–2791, 2008.
- * Extensive review of experiments and theoretical description of single-molecule electron transfer in electrochemical environments.
- [8] Birdi KS. *Scanning probe microscopes : applications in science and technology*. CRC Press, 2003.
- [9] Wiesendanger R. *Scanning probe microscopy and spectroscopy : methods and applications*. Cambridge University Press, 1994.
- [10] Binnig G, Rohrer H, Gerber C, and Weibel E. Surface Studies by Scanning Tunneling Microscopy. *Phys Rev Lett*, **49**(1):57–61, 1982.
- [11] Tao NJ. Probing potential-tuned resonant tunneling through redox molecules with scanning tunneling microscopy. *Phys Rev Lett*, **76**(21):4066–4069, 1996.
- [12] Xu B. Measurement of Single-Molecule Resistance by Repeated Formation of Molecular Junctions. *Science (80-)*, **301**(5637):1221–1223, 2003.
- [13] Haiss W, van Zalinge H, Higgins SJ, Bethell D, Höbenreich H, Schiffrin DJ, and Nichols RJ. Redox State Dependence of Single Molecule Conductivity. *J Am Chem Soc*, **125**(50):15294–15295, 2003.
- [14] Pires E, Macdonald JE, and Elliott M. Chain length and temperature dependence of alkanedithiol molecular conductance under ultra high vacuum. *Nanoscale*, **5**(19):9397–403, 2013.
- [15] Inkpen MS, Lemmer M, Fitzpatrick N, Milan DC, Nichols RJ, Long NJ, and Albrecht T. New Insights into Single-Molecule Junctions Using a Robust, Unsupervised Approach to Data Collection and Analysis. *J Am Chem Soc*, **137**(31):9971–9981, 2015.
- * Development of an automated and robust approach to STM current-distance data collection and analysis for single-molecule junctions.
- [16] Guo S, Hihath J, Díez-Pérez I, and Tao N. Measurement and statistical analysis of single-molecule current-voltage characteristics, transition voltage spectroscopy, and tunneling barrier height. *J Am Chem Soc*, **133**(47):19189–19197, 2011.
- [17] Haiss W, Nichols RJ, van Zalinge H, Higgins SJ, Bethell D, and Schiffrin DJ. Measurement of single molecule conductivity using the spontaneous formation of molecular wires. *Phys Chem Chem Phys*, **6**(17):4330, 2004.
- [18] Nichols RJ, Haiss W, Higgins SJ, Leary E, Martin S, and Bethell D. The experimental determination of the conductance of single molecules. *Phys Chem Chem Phys*, **12**(12):2801–15, 2010.
- [19] Chappell S, Brooke C, Nichols RJ, Kershaw Cook LJ, Halcrow M, Ulstrup J, and Higgins SJ. Evidence for a hopping mechanism in metal—single molecule—metal junctions involving conjugated metal-terpyridyl complexes; potential-dependent conductances of complexes [M(pyterpy) 2] 2+ (M = Co and Fe; pyterpy = 4'-(pyridin-4-yl)-2,2':6',2''-terpyridine. *Faraday Discuss*, **193**:113–131, 2016.
- [20] Sakai K, Okada Y, Uemura T, Tsurumi J, Häusermann R, Matsui H, Fukami T, Ishii H, Kobayashi N, Hirose K, and Takeya J. The emergence of charge coherence in soft molecular organic semiconductors via the suppression of thermal fluctuations. *NPG Asia Mater*, **8**(3):e252, 2016.
- [21] Lambert C, Nöll G, and Schelter J. Bridge-mediated hopping or superexchange electron-transfer processes in bis(triarylamine) systems. *Nat Mater*, **1**(1):69–73, 2002.
- [22] Li X, He J, Hihath J, Xu B, Lindsay SM, and Tao N. Conductance of Single Alkanedithiols: Conduction Mechanism and Effect of Molecule-Electrode Contacts. *J Am Chem Soc*, **128**(6):2135–2141, 2006.
- [23] Datta S. *Quantum Transport: Atom to Transistor*. Cambridge University Press, 2005.
- [24] Pobelov IV, Li Z, and Wandlowski T. Electrolyte gating in redox-active tunneling junctions—an electrochemical STM approach. *J Am Chem Soc*, **130**(47):16045–54, 2008.
- [25] Zhang J, Chi Q, Hansen AG, Jensen PS, Salvatore P, and Ulstrup J. Interfacial electrochemical electron transfer in biology - Towards the level of the single molecule. *FEBS Lett*, **586**(5):526–535, 2012.
- [26] Kuznetsov AM, Sommer-Larsen P, and Ulstrup J. Resonance and environmental fluctuation effects in STM currents through large adsorbed molecules. *Surf Sci*, **275**(1-2):52–64, 1992.
- [27] Kuznetsov AM and Ulstrup J. Mechanisms of in Situ Scanning Tunneling Microscopy of Organized Redox Molecular Assemblies. *J Phys Chem A*, **104**(49):11531–11540, 2000.
- [28] Sumi H, Hori Y, and Mukai K. Marcus parabola and reorganization energies associated with redox change of electron-transfer proteins, detected by VI characteristics of STM currents. *J Electroanal Chem*, **592**(1):46–62, 2006.
- [29] Bâldea I. Extending the Newns-Anderson model to allow nanotransport studies through molecules with floppy degrees of freedom. *Europhys Lett*, **99**(4):47002, 2012.
- [30] Rzeźnicka II, Worpel GW, Bonn M, van der Horst MA, Hellingwerf KJ, Matsunaga S, Yamada T, and Kawai M. Observation of photoactive yellow protein anchored to a modified Au(111) surface by scanning tunneling microscopy. *Chem Phys Lett*, **472**(1-3):113–117, 2009.
- [31] Friis E, Andersen J, Madsen L, Möller P, and Ulstrup J. In situ STM and AFM of the copper protein Pseudomonas aeruginosa azurin. *J Electroanal Chem*, **431**(1):35–38, 1997.
- [32] Chi Q, Zhang J, Nielsen J, Friis E, Chorkendorff I, Canters G, Andersen J, and Ulstrup J. Molecular monolayers and interfacial electron transfer of Pseudomonas aeruginosa azurin on Au (111). *J Am Chem Soc*, **122**(17):4047–4055, 2000.
- ** The first study of an electrochemically functional re-

- dox protein monolayer at single-crystal metal electrodes. A wide range of techniques including EC-STM.
- [33] Chi Q, Farver O, and Ulstrup J. Long-range protein electron transfer observed at the single-molecule level: In situ mapping of redox-gated tunneling resonance. *PNAS*, **102**(45):16203–8, 2005.
- [34] Artés JM, Díez-Pérez I, and Gorostiza P. Transistor-like behavior of single metalloprotein junctions. *Nano Lett*, **12**(6):2679–84, 2012.
- [35] Artés JM, López-Martínez M, Díez-Pérez I, Sanz F, and Gorostiza P. Conductance switching in single wired redox proteins. *Small*, **10**(13):2537–2541, 2014.
- ** Observation of redox switching events in single protein molecules has been reported in spontaneously formed single-wire protein junctions in an electrochemical environment.
- [36] Baldacchini C, Kumar V, Bizzarri AR, and Cannistraro S. Electron tunnelling through single azurin molecules can be on/off switched by voltage pulses. *Appl Phys Lett*, **106**(18), 2015.
- * STM imaging of single azurin molecules adsorbed on Au indicates that their tunnelling conductance can be reversibly switched by applying voltage pulse to the STM tip, resulting in long-lived electron trapping on the molecule.
- [37] Friis EP, Andersen JET, Kharkats YI, Kuznetsov AM, Nichols RJ, Zhang JD, and Ulstrup J. An approach to long-range electron transfer mechanisms in metalloproteins: In situ scanning tunneling microscopy with submolecular resolution. *PNAS*, **96**(4):1379–1384, 1999.
- [38] Wang J, Zhang L, Hu C, Liu Q, Hou Y, Zhang X, and Lu Q. Sub-molecular features of single proteins in solution resolved with scanning tunneling microscopy. *Nano Res*, **9**(9):2551–2560, 2016.
- ** By optimising experimental parameters it has proved possible to obtain sub-molecular resolution of a single streptavidin protein molecule in solution.
- [39] Della Pia EA, Chi Q, Macdonald JE, Ulstrup J, Jones DD, and Elliott M. Fast electron transfer through a single molecule natively structured redox protein. *Nanoscale*, **4**(22):7106–13, 2012.
- [40] Ouyang W and Subotnik JE. The dynamics of charge transfer with and without a barrier: A very simplified model of cyclic voltammetry. *J Chem Phys*, **146**(17):174103, 2017.
- [41] Bâldea I. Important insight into electron transfer in single-molecule junctions based on redox metalloproteins from transition voltage spectroscopy. *J Phys Chem C*, **117**(48):25798–25804, 2013.
- ** An interesting alternative description to the two-level model of how a protein mediates electron transport between an STM tip and substrate.
- [42] Corni S. A theoretical study of the electrochemical gate effect in an STM-based biomolecular transistor. *IEEE Trans Nanotechnol*, **6**(5):561–570, 2007.
- [43] Leary E, Höbenreich H, Higgins S, van Zalinge H, Haiss W, Nichols RJ, Finch C, Grace I, Lambert C, McGrath R, and Smerdon J. Single-Molecule Solvation-Shell Sensing. *Phys Rev Lett*, **102**(8):1–4, 2009.
- [44] Fatemi V, Kamenetska M, Neaton JB, and Venkataraman L. Environmental control of single-molecule junction transport. *Nano Lett*, **11**(5):1988–92, 2011.
- [45] Kotiuga M, Darancet P, Arroyo CR, Venkataraman L, and Neaton JB. Adsorption-Induced Solvent-Based Electrostatic Gating of Charge Transport through Molecular Junctions. *Nano Lett*, **15**(7):4498–4503, 2015.
- * Experimental and theoretical advance in understanding the electrostatic effects of solvent environment on the conductance of single-molecule junctions.
- [46] Kastlunger G and Stadler R. Charge localization on a redox-active single-molecule junction and its influence on coherent electron transport. *Phys Rev B - Condens Matter Mater Phys*, **88**(3):1–9, 2013.
- [47] Seyed SS, Waskasi MM, and Matyushov DV. Theory and Electrochemistry of Cytochrome c. *J Phys Chem B*, **121**(19):4958–4967, 2017.
- * An interesting theoretical model to explain the occurrence of low reorganisation energy determined from electrochemical experiments on cytochrome c.
- [48] Matyushov DV. Protein electron transfer: is biology (thermo)dynamic? *J Phys Condens Matter*, **27**(47):473001, 2015.
- [49] Matyushov DV. Protein electron transfer: Dynamics and statistics. *J Chem Phys*, **139**(2), 2013.
- [50] Martin DR, Lebard DN, and Matyushov DV. Coulomb soup of bioenergetics: Electron transfer in a bacterial bc 1 complex. *J Phys Chem Lett*, **4**(21):3602–3606, 2013.
- [51] Osorio HM, Catarelli S, Cea P, Gluyas JBG, Hartl FH, Higgins SJ, Leary E, Low PJ, Martín S, Nichols RJ, Tory J, Ulstrup J, Vezzoli A, Milan DC, and Zeng Q. Electrochemical Single-Molecule Transistors with Optimized Gate Coupling. *J Am Chem Soc*, **137**(45):14319–14328, 2015.
- ** Electrochemical gating mediated by ionic liquids is shown to be far more effective than for aqueous media. Using the two-step model for charge transfer across the molecule in EC-STM an effective gate coupling constant of unity was found, compared with 0.2 in aqueous solution.
- [52] Amdursky N, Marchak D, Sepunaru L, Pecht I, Sheves M, and Cahen D. Electronic Transport via Proteins. *Adv Mater*, **26**(42):7142–7161, 2014.
- [53] Bostick CD, Mukhopadhyay S, Sheves M, Cahen D, and Lederman D. Protein bioelectronics : a review of what we do and do not know. *ArXiv Prepr*, page 1702.05028, 2017.
- * Summarises basic mechanisms of protein electron transfer or transport, putting them in the context of present understanding. Describes practical means to anchor and measure electronic properties of proteins on solids. Looks at how biological activity of immobilised proteins is of use in bioelectronics.
- [54] Wu H, Feng X, Kieviet BD, Zhang K, Zandvliet HJW, Canters GW, Schön PM, and Vancso GJ. Electrochemical atomic force microscopy reveals potential stimulated height changes of redox responsive Cu-azurin on gold. *Eur Polym J*, **83**:529–537, 2016.
- * EC-AFM studies of single molecules show that the height of Cu-azurin can be reversibly changed by 0.32nm upon redox switching, whilst Zn-azurin is unaltered.
- [55] Giannotti MI, Cabeza De Vaca I, Artés JM, Sanz F, Guallar V, and Gorostiza P. Direct Measurement of the Nanomechanical Stability of a Redox Protein Active Site and Its Dependence upon Metal Binding. *J Phys Chem B*, **119**(36):12050–12058, 2015.
- [56] Baldacchini C, Bizzarri AR, and Cannistraro S. Electron transfer, conduction and biorecognition properties of the redox metalloprotein Azurin assembled onto inorganic substrates. *Eur Polym J*, **83**:407–427, 2016.
- [57] Georgakilas V, Otyepka M, Bourlinos AB, Chandra V, Kim N, Kemp KC, Hobza P, Zboril R, and Kim KS. Functionalization of Graphene: Covalent and Non-Covalent Approaches, Derivatives and Applications. *Chem Rev*, **112**(11):6156–6214, 2012.
- [58] De Leo F, Magistrato A, and Bonifazi D. Interfacing proteins with graphitic nanomaterials: from spontaneous attraction to tailored assemblies. *Chem Soc Rev*, **44**(19):6916–6953, 2015.
- [59] Halder A, Zhang M, and Chi Q. Electroactive and biocompatible functionalization of graphene for the development of biosensing platforms. *Biosens Bioelectron*, **87**:764–771, 2017.
- [60] Chi Q, Ford MJ, Halder A, Hush NS, Reimers JR, and Ulstrup J. Sulfur ligand mediated electrochemistry of gold surfaces and nanoparticles: What, how, and why. *Curr Opin Electrochem*, **1**(1):7–15, 2017.
- [61] Wen J, Xu Y, Li H, Lu A, and Sun S. Recent applications of carbon nanomaterials in fluorescence biosensing and bioimaging. *Chem Commun*, **51**(57):11346–11358, 2015.
- [62] Ping J, Xi J, Saven JG, Liu R, and Johnson ATC. Quantifying the effect of ionic screening with protein-decorated graphene transistors. *Biosens Bioelectron*, **89**:689–692, 2017.
- [63] Choi Y, Olsen TJ, Sims PC, Moody IS, Corso BL, Dang MN, Weiss GA, and Collins PG. Dissecting single-molecule signal transduction in carbon nanotube circuits with protein engineering. *Nano Lett*, **13**(2):625–31, 2013.
- ** Advance in understanding mechanism of single walled carbon nanotube-based FET signal transduction through conformationally driven electrostatic gating by an attached lysozyme molecule. Gating was related to charged side

chains located close to the SWNT demonstrating a broad applicability.

- [64] Münzer AM, Michael ZP, and Star A. Carbon nanotubes for the label-free detection of biomarkers. *ACS Nano*, **7**(9):7448–7453, 2013.
- [65] Vijayaraghavan A, Oron-Carl M, Blatt S, Vijayaraghavan A, Blatt S, Weissenberger D, Weissenberger D, Oron-Carl M, Hennrich F, Hennrich F, Gerthsen D, Hahn H, Gerthsen D, Hahn H, Krupke R, and Krupke R. Ultra-large-scale directed assembly of single-walled carbon nanotube devices. Supporting Information. *Nano Lett*, **7**(6):1556–1560, 2007.